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Long-Term *Rasamsonia argillacea* Complex Species Colonization Revealed by PCR Amplification of Repetitive DNA Sequences in Cystic Fibrosis Patients

Abdelmounaim Mouhajir,[a,b] Olivier Matray,[c,d] Sandrine Giraud,[a] Laurent Mély,[e] Christophe Marguet,[f] Isabelle Sermet-Gaudelus,[g] Solène Le Gal,[a,h] Franck Labbé,[i] Christine Person,[j] Françoise Troussier,[k] Jean-Jacques Ballet,[c] Gilles Gargala,[c,d] Rachid Zouhair,[b] Marie-Elisabeth Bougnoux,[l] Jean-Philippe Bouchara,[a,m] Loïc Favennec[c,d]

L'UNAM Université, Université d'Angers, Groupe d'Etude des Interactions Hôte-Pathogène, Angers, France[a]; University Moulay Ismail, Faculty of Sciences, Meknes, Morocco[b]; Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire, Rouen, France[c]; Normandie University, UNIROUEN, Rouen, France[d]; Centre de Ressources et de Compétences en Mucoviscidose, Hôpital Renée Sabran, Giens, France[e]; Centre de Ressources et de Compétences en Mucoviscidose, Service de Pédiatrie, Hôpital Charles Nicolle, Rouen, France[f]; Centre de Ressources et de Compétences en Mucoviscidose, Unité de Pédiatrie, Hôpital Necker-Enfants Malades, Paris, France[g]; Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire, Brest, France[h]; Laboratoire de Microbiologie, Hôpital Jacques Monod, Le Havre, France[i]; Centre de Ressources et de Compétences en Mucoviscidose Adultes, Service de Pneumologie, Centre Hospitalier Universitaire, Angers, France[j]; Centre de Ressources et de Compétences en Mucoviscidose Enfants, Service de Pédiatrie, Centre Hospitalier Universitaire, Angers, France[k]; Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire Necker-Enfants Malades, Paris, France[l]; Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire, Angers, France[m]

The aim of this work was to document molecular epidemiology of *Rasamsonia argillacea* species complex isolates from cystic fibrosis (CF) patients. In this work, 116 isolates belonging to this species complex and collected from 26 CF patients and one patient with chronic granulomatous disease were characterized using PCR amplification assays of repetitive DNA sequences and electrophoretic separation of amplicons (rep-PCR). Data revealed a clustering consistent with molecular species identification. A single species was recovered from most patients. *Rasamsonia aegroticola* was the most common species, followed by *R. argillacea sensu stricto* and *R. piperina*, while *R. eburnea* was not identified. Of 29 genotypes, 7 were shared by distinct patients while 22 were patient specific. In each clinical sample, most isolates exhibited an identical genotype. Genotyping of isolates recovered from sequential samples from the same patient confirmed the capability of *R. aegroticola* and *R. argillacea* isolates to chronically colonize the airways. A unique genotype was recovered from two siblings during a 6-month period. In the other cases, a largely dominant genotype was detected. Present results which support the use of rep-PCR for both identification and genotyping for the *R. argillacea* species complex provide the first molecular evidence of chronic airway colonization by these fungi in CF patients.

Rasamsonia argillacea, which was first reported in clinical practice as a *Penicillium* species, then reclassified in the genus *Geosmithia*, and finally reassigned in 2013 to the new genus *Rasamsonia*, is now considered an emerging pathogen (1). Since its first report in humans as *Penicillium emersonii* (2), there has been an increasing number of cases reported in both dogs (3, 4, 5) and humans (6). Nevertheless, it is likely that the number of human infections caused by this fungus has long been underestimated because of the lack of specificity of its morphological features and to subsequent misidentifications with some *Penicillium* or *Paecilomyces* species.

Only six species were previously recognized in the genus *Rasamsonia* described in 2012, i.e., *R. argillacea*, *R. composticola*, *R. brevistipitata*, *R. byssochlamydoides*, *R. cylindrospora*, and *R. emersonii* (7). However, multilocus sequence analysis of several *R. argillacea* isolates revealed a clustering which superimposed some phenotypic differences. Therefore, *R. argillacea* is now considered a species complex comprising four distinct species: *R. argillacea sensu stricto*, *R. piperina*, *R. aegroticola*, and *R. eburnea* (8).

Infections caused by species of the *R. argillacea* complex have been reported in various clinical contexts in human. Chronic granulomatous disease (CGD) (9, 10, 11) and cystic fibrosis (CF) (12, 13, 14, 15, 16, 17) are the major underlying clinical conditions. *Rasamsonia* infections may also occur in bone marrow transplant recipients (18, 19) but also may occur in the absence of any predisposing factors, as evidenced by the pulmonary and aortic graft infection reported by Doyon et al. (20) in an immunocompetent individual.

The importance of the identification of these fungi is underlined by their pathogenicity, which was documented in various clinical settings, including CGD patients and bone marrow transplant recipients who developed pneumonia and/or disseminated infections (9, 10, 11, 18, 19) or patients with tuberculosis who suffered from fungal balls in the lung (21). In CF patients, all species of the *R. argillacea* complex except *R. eburnea* have been

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Address correspondence to Loïc Favennec, loic.favennec@chu-rouen.fr.

A.M. and O.M. contributed equally to this work, as did J.-P.B. and L.F.

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reported to colonize the airways (8). While no clear relationship was established in the CF context between the colonization of the airways by these fungi and a clinical or functional deterioration, the recent report of *R. argillacea* pathogenicity in one patient with CF prompts further investigation (14). In addition, differences between these closely related species in their antifungal susceptibility profiles further emphasize the need for an accurate identification of the fungus for optimal therapy (1, 8). However, members of the genus *Rasamsonia*, particularly those belonging to the *R. argillacea* species complex, are difficult to identify precisely in a routine clinical setting because of the lack of specific traits in their macroscopic and microscopic morphology. Therefore, sequencing of the internal transcribed spacer (ITS) 1 and 2 regions of ribosomal DNA, or of part of the beta-tubulin or calmodulin genes, is required for accurate species identification within the *R. argillacea* complex (7, 8). Nevertheless, these methods are time-consuming and do not allow strain differentiation for epidemiological purposes. In this context, this work was designed to evaluate the interest of PCR amplification of repetitive DNA sequences (rep-PCR) for species and strain delineation within this species complex. The study was conducted using a large panel of multiple (originating from the same clinical sample) or sequential (originating from successive samples from the same patient) isolates from CF patients and from one acutely infected CGD patient. Sequencing results were compared to those obtained using the semiautomated Diversilab system (bioMérieux) based on PCR amplification of repetitive sequences followed by microelectrophoretic separation of the amplicons and size analysis using dedicated software.

MATERIALS AND METHODS

Clinical isolates and culture conditions. One hundred nine isolates collected from 1996 to December 2012 from sputum samples from 24 patients with CF that had been followed up in six geographically distant hospitals in France were included in this study. Three isolates recovered from two CF patients followed up in the United Kingdom (1 isolate) and in Greece (2 isolates), as well as four isolates recovered from one French patient with CGD, were studied in parallel.

Isolates were kept frozen at −20°C until use. After thawing, they were plated on yeast extract-peptone-dextrose (YPD) agar plates supplemented with chloramphenicol (0.5 g/liter). After incubation at 37°C for 7 days, the aerial mycelium was scraped from the agar surface and then inoculated in YPD broth, which was incubated for 10 days at 37°C without stirring. Mycelium was finally harvested and ground in liquid nitrogen.

Species identification. Apart from 16 of the isolates studied which had been previously identified by ITS 1 and 2 regions and beta-tubulin and calmodulin gene sequencing (8, 13), species identification was performed by PCR amplification of part of the beta-tubulin gene (TUB locus). After grinding the mycelium in liquid nitrogen, DNA was extracted using the DNeasy plant minikit (Qiagen, Courtabeuf, France), and the TUB locus was amplified as previously described (22). The amplified products were then purified using the NucleoSpin extract II kit (Macherey-Nagel, Hoerd, France) and sequenced in both directions. The obtained sequences were analyzed by individual BLASTn searches using the NCBI BLAST database. Ninety-seven to 100% sequence similarity to isolates previously analyzed by Houbraken et al. (8) was used for precise species identification within the *R. argillacea* complex.

Semiautomated repetitive sequence-based PCR and data analysis. Fungal DNA was extracted from mycelial homogenates using the Mo Bio UltraClean preparation kit (Ozyme, Saint-Quentin en Yvelines, France) according to the manufacturer's recommendations except for the protein precipitation step, the duration of which was extended overnight at 4°C. DNA was quantified using a NanoDrop spectrometer (Thermo Fisher Scientific, Illkirsch, France) for a target value of ≥25 ng/ml. PCR amplification of repetitive DNA sequences was realized using the DiversiLab fungal kit (bioMérieux) as described previously (23). Amplicons were separated by capillary electrophoresis on an Agilent 2100 bioanalyzer (Massy, France). Single electrophoretic profiles obtained for each isolate were compared using the DiversiLab Healthcare software (version 3.41). The phylogenetic analysis was based on dendrograms generated by the DiversiLab software using the unweighted pair group method with arithmetic means (UPGMA). Isolates sharing less than 95% similarity were considered to belong to distinct rep-PCR genotypes which were numbered according to their order in the dendrogram.

Accession number(s). The sequences described were submitted to GenBank under the accession numbers given in Table 1.

RESULTS

Species identification. In addition to the 16 isolates already identified at the species level by Houbraken et al. (8) or Giraud et al. (13), 96 isolates also recovered from sputum samples from CF patients were analyzed by TUB sequencing (Table 1). Among all of these isolates, *R. aegroticola* was by far the most common species, representing 80 isolates, followed by *R. argillacea sensu stricto* and *R. piperina*, which accounted for 27 and 5 isolates, respectively. *Rasamsonia eburnea* was never identified. Only one patient (P22) carried successively two distinct species (i.e., *R. argillacea* in 2006 and *R. aegroticola* in 2009), although up to 18 isolates were studied for other patients. The four isolates from patient P27 with CGD were also identified as *R. argillacea sensu stricto*.

rep-PCR was conducted on the whole set of isolates using the mold DNA fingerprinting primer kit, and from the obtained electrophoretic profiles, the isolates were categorized as indistinguishable, closely related, or different according to the interpretative criteria provided by the manufacturer. An example of isolates sharing the same electrophoretic profile and therefore belonging to the same rep-PCR genotype is presented in Fig. 1A with *R. aegroticola* AU 49708718 and AU 49709968 isolates obtained from two successive sputum samples from patient P17. Figure 1B shows an example of closely related isolates, i.e., differing only by 1 or 2 peaks, with *R. argillacea sensu stricto* AU 080618744-01/3 and AU 125955913-01/3 isolates collected from distinct sputum samples from patient P24. An example of genetically different isolates with electrophoretic profiles differing by at least 3 peaks is shown in Fig. 1C, with *R. piperina* AU 110334223-01 and AU 080120123-01 isolates recovered from two patients (P19 and P20) followed up in geographically distant hospitals.

By rep-PCR, the isolates were grouped into three clusters strictly superimposed with species identification by ITS 1 and 2 regions or beta-tubulin gene sequencing (Table 1). Figure S1 in the supplemental material shows the dendrogram constructed by the DiversiLab software from the electrophoretic profiles of the amplicons generated by rep-PCR analysis of the different isolates. All 80 isolates identified by TUB sequencing as *R. aegroticola* were grouped in the same cluster, clearly separated from the 31 *R. argillacea sensu stricto* isolates which constitute another cluster and from the 5 *R. piperina* isolates which were grouped in a third cluster.

In addition, the distribution of *Rasamsonia* species according to geographic origin of the patients or isolates was investigated. In contrast to *R. piperina*, the less common species, which was detected only from patients followed up in Giens and Rouen hospitals, *R. argillacea* and *R. aegroticola* were almost equally recovered from the different locations (Fig. 2). For example, *R. aegroticola*

TABLE 1 Species and genotype of studied clinical *Rasamsonia* isolates[d]

Patient no. and sampling date (yr-mo-day)	Isolate no.	Geographic origin	rep-PCR genotype	Species identification	GenBank accession no.[a]
P1					
2008-02-15	080120121-01	Giens, France	13	*R. aegroticola*	KT873369
2008-10-02	080578563-01		16	*R. aegroticola*	GU165734[b]
2009-08-03	090482165-01		13	*R. aegroticola*	KT873370
2009-09-22	090602132-01		13	*R. aegroticola*	KT873365
2010-10-04	100640715-01/2		13	*R. aegroticola*	KT873363
P2					
2011-04-13	110201721-01	Rouen, France	16	*R. aegroticola*	KT873374
P3					
2011-02-07	110089119-01	Angers, France	16	*R. aegroticola*	KT873379
2011-11-21	115371503-51		16	*R. aegroticola*	KT873346
P4					
2011-06-22	110349466-02	Paris, France	15	*R. aegroticola*	KT873351
2011-10-26	1110M260319		15	*R. aegroticola*	KT873350
2012-01-18	1201M180296		15	*R. aegroticola*	KT873348
P5					
2011-10-26	1110M262314	Paris, France	15	*R. aegroticola*	KT873352
2012-01-18	1201M180402		15	*R. aegroticola*	KT873347
	1201M180404		15	*R. aegroticola*	KT873355
2012-03-15	1203M150283		15	*R. aegroticola*	KT873354
2012-03-22	1203M220242		15	*R. aegroticola*	KT873356
	1203M220245		15	*R. aegroticola*	KT873353
P6					
2009-11-25	090694857-01	Angers, France	15	*R. aegroticola*	KT873360
2010-02-17	100112439-01		15	*R. aegroticola*	KT873358
P7					
2009-11-03	090676598-01	Giens, France	15	*R. aegroticola*	KT873357
2010-01-05	100037113-01		15	*R. aegroticola*	KT873359
2010-10-07	100663651-01		16	*R. aegroticola*	KT873349
P8					
2010-07-07	100421474-01	Angers, France	15	*R. aegroticola*	KT873371
P9					
2010-06-22	100563919-02	Rouen, France	17	*R. aegroticola*	KT873384
2010-12-16	110098614-01		16	*R. aegroticola*	KT873372
2011-01-07	120138251-01		17	*R. aegroticola*	KT873385
2011-03-08	110201710-01		16	*R. aegroticola*	KT873373
2011-03-30	110201720-01		16	*R. aegroticola*	KT873375
2011-05-13	120138254-01		15	*R. aegroticola*	KT873382
2011-06-14	110245715-01		16	*R. aegroticola*	KT873381
2011-08-09	110310506-01		16	*R. aegroticola*	KT873380
2011-09-09	110310508-01		13	*R. aegroticola*	KT873378
2011-12-02	110358956-01		15	*R. aegroticola*	KT873376
2012-08-29	120167555-01		16	*R. aegroticola*	KT873377
P10					
2011-05-05	110235102-01	Rouen, France	10	*R. aegroticola*	KT873388
2011-08-25	11081139		12	*R. aegroticola*	KT873389
2011-11-14	110349459-01		18	*R. aegroticola*	KT873390
2012-03-14	120097976-01		10	*R. aegroticola*	KT873391
2012-04-19	120138238-01		10	*R. aegroticola*	KT873392
2012-06-21	12060929		10	*R. aegroticola*	KT873393
P11					
2003	IHEM 20178	Brest, France	11	*R. aegroticola*	KT873394

(Continued on following page)

TABLE 1 (Continued)

Patient no. and sampling date (yr-mo-day)	Isolate no.	Geographic origin	rep-PCR genotype	Species identification	GenBank accession no.[a]
P12					
2011-08-03	UOA/HCPF 13695	Athens, Greece	19	*R. aegroticola*	KT873387
2012-07-18	UOA/HCPF 14683a		19	*R. aegroticola*	KT873386
P13					
1991	NCPF 2801	UK	19	*R. aegroticola*	JX272998[c]
P14					
2005-08-31	40505666/1	Giens, France	20	*R. aegroticola*	GU165728[b]
	40505666/2		20	*R. aegroticola*	KT873361
	40505666/3		19	*R. aegroticola*	KT873364
	40505666/4		20	*R. aegroticola*	KT873368
	40505666/5		23	*R. aegroticola*	KT873366
2007-11-07	40709168		20	*R. aegroticola*	GU165729[b]
P15					
2007-04-20	070116422-01	Rouen, France	23	*R. aegroticola*	GU165736[b]
2007-06-25	070116430-01		21	*R. aegroticola*	GU165737[b]
2007-11-12	070116440-01		21	*R. aegroticola*	GU165738[b]
2008-10-01	080677948-01		21	*R. aegroticola*	GU165739[b]
2008-12-08	100608593-01		23	*R. aegroticola*	KT873399
2009-03-16	090213107-01	Rouen, France	21	*R. aegroticola*	KT873411
2009-06-10	100608601-01		21	*R. aegroticola*	KT873400
2009-08-27	100608602-01		21	*R. aegroticola*	KT873410
2009-10-07	120137893-01		21	*R. aegroticola*	KT873408
2009-10-15	100608604-01		23	*R. aegroticola*	KT873415
2009-11-26	11031051		21	*R. aegroticola*	KT873416
2010-01-04	100608605-01		21	*R. aegroticola*	KT873409
2010-04-14	100608607-01		19	*R. aegroticola*	KT873402
2010-05-17	100608609-01		21	*R. aegroticola*	KT873404
2010-12-13	110098612-01		21	*R. aegroticola*	KT873396
P16					
2011-06-22	110251637-01	Le Havre, France	22	*R. aegroticola*	KT873367
P17					
1996	49701666	Giens, France	21	*R. aegroticola*	KT873414
1997-09-25	49707800		19	*R. aegroticola*	DQ317583[b]
					DQ317584[b]
1997-10-27	49708718		21	*R. aegroticola*	KT873405
1997-12-11	49709968		21	*R. aegroticola*	KT873398
1998-01-21	49800813/1	Giens, France	21	*R. aegroticola*	KT873403
	49800813/2		21	*R. aegroticola*	KT873406
	49800813/3		21	*R. aegroticola*	KT873395
	49800813/4		21	*R. aegroticola*	KT873401
	49800813/5		21	*R. aegroticola*	KT873413
	49800813/6		21	*R. aegroticola*	KT873412
	49800813/7		21	*R. aegroticola*	KT873407
2001	IHEM 18732		21	*R. aegroticola*	KT873397
P18					
2011-01-18	120138249-01	Rouen, France	24	*R. aegroticola*	KT873383
P19					
2011-10-12	110334223-01	Rouen, France	27	*R. piperina*	KT873447
P20					
2007-10-09	40708324	Giens, France	26	*R. piperina*	GU165731[b]
2008-01-07	080033462-01		25	*R. piperina*	KT873444
2008-02-20	080120123-01		29	*R. piperina*	GU165733[b]

(Continued on following page)

TABLE 1 (Continued)

Patient no. and sampling date (yr-mo-day)	Isolate no.	Geographic origin	rep-PCR genotype	Species identification	GenBank accession no.[a]
P21					
2010-11-05	120140359-01	Rouen, France	28	*R. piperina*	KT873445
P22					
2006-12-14	40608890	Angers, France	3	*R. argillacea*	GU165726[b]
2009-11-03	090654817-01		14	*R. aegroticola*	KT873362
P23					
2011-10-26	1110M260218	Paris, France	5	*R. argillacea*	KT873428
2012-03-14	1203M140384		5	*R. argillacea*	KT873427
P24					
2007-09-18	40707439	Angers, France	2	*R. argillacea*	GU165722[b]
2007-11-29	070069943-01		9	*R. argillacea*	KT873424
2007-12-05	070078695-01		4	*R. argillacea*	KT873426
2008-02-26	080118059-01/1		4	*R. argillacea*	GU165724[b]
	080118059-01/2		1	*R. argillacea*	KT873431
	080118059-01/3		4	*R. argillacea*	KT873422
2008-04-15	080220281-01/1		4	*R. argillacea*	KT873435
2008-09-22	080536592-01		4	*R. argillacea*	GU165725[b]
2008-11-03	080618744-01/1		1	*R. argillacea*	KT873430
	080618744-01/3		1	*R. argillacea*	KT873436
	080618744-01/4		9	*R. argillacea*	KT873443
	080618744-01/5		1	*R. argillacea*	KT873425
2009-06-15	090361642-01		1	*R. argillacea*	KT873432
2010-02-23	100122619-01		1	*R. argillacea*	KT873434
2012-12-18	125955913-01/1	Angers, France	1	*R. argillacea*	KT873439
	125955913-01/2		1	*R. argillacea*	KT873440
	125955913-01/3		1	*R. argillacea*	KT873441
	125955913-01/4		1	*R. argillacea*	KT873442
P25					
2009-02-01	090143735-01	Rouen, France	8	*R. argillacea*	JX273009[c]
P26					
2011-09-02	110310498-01	Rouen, France	3	*R. argillacea*	KT873429
2011-12-22	11121060		6	*R. argillacea*	KT873438
2012-01-03	120030197-01		3	*R. argillacea*	KT873421
2012-02-10	120138261-01		3	*R. argillacea*	KT873437
2012-08-17	120143501-01		3	*R. argillacea*	KT873423
P27					
2010-03-22	1003M220610	Paris, France	7	*R. argillacea*	KT873419
2010-03-24	1003M240080		7	*R. argillacea*	KT873418
2010-03-25	1003M250191		7	*R. argillacea*	KT873417
	1003M250160		7	*R. argillacea*	KT873420

[a] All isolates were identified at the species level in the present study unless otherwise indicated. GenBank accession numbers correspond to the nucleotide sequence of ITS 1 and 2 regions of rRNA genes for isolates identified by Giraud et al. (13) or of part of the beta-tubulin gene (TUB locus) for the other isolates.
[b] Sequences previously identified by Giraud et al. (13).
[c] Sequences previously identified by Houbraken et al. (8).
[d] All isolates were recovered from sputum samples from patients with cystic fibrosis, except isolates from patient P27, who suffered from chronic granulomatous disease.

was identified from 4/6, 4/5, 2/4, and 6/10 patients from Angers, Giens, Paris-Necker, and Rouen hospitals, respectively.

Genotype differentiation and patterns of colonization. rep-PCR also permitted strain delineation within the *R. argillacea* species complex. Fifteen genotypes were identified among the 80 *R. aegroticola* isolates. Nine genotypes were identified for *R. argillacea sensu stricto*, and all five *R. piperina* isolates were identified as distinct genotypes.

The 80 isolates identified as *R. aegroticola* were recovered from 68 sputum samples collected from 19 CF patients (Table 1). Only one sputum sample was available for six of the patients, but at least two culture-positive samples were collected for the others. Apart from patient P22, who successively carried two distinct species, rep-PCR analysis showed that 5 of the patients were colonized by a single genotype conserved over time, while a dominant genotype was identified in successive samples from the seven remaining patients, associated with one to three other genotypes of the same species found occasionally (Fig. 3). Interestingly, *R. aegroticola*

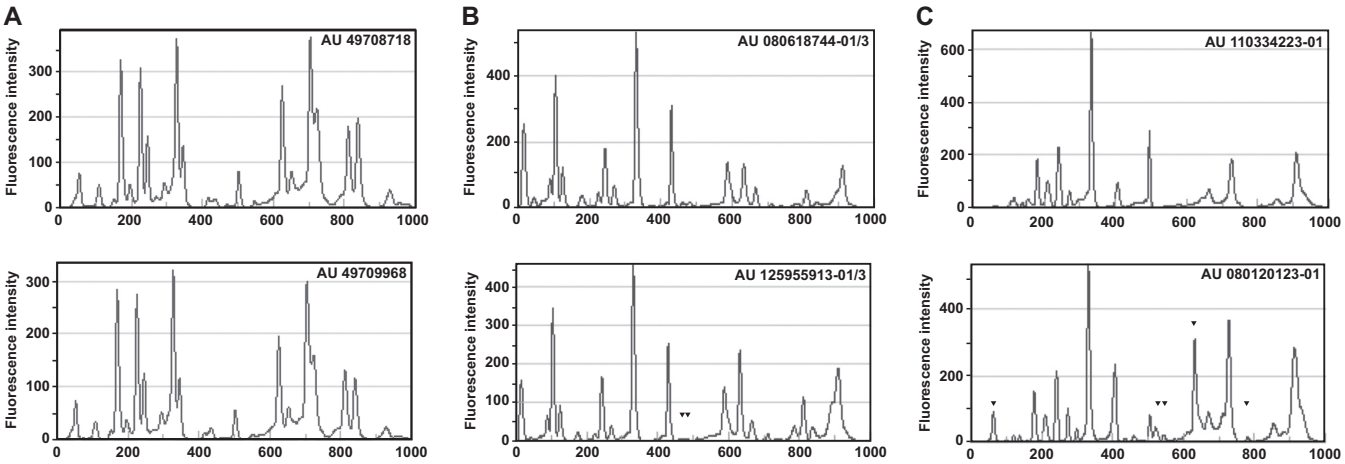

FIG 1 Typical examples of electrophoretic profiles obtained by rep-PCR using the mold DNA fingerprinting primer kit. (A) Identical profiles with *R. aegroticola* AU 49708718 and AU 49709968 isolates obtained from two successive sputum samples from patient P17. (B) Related profiles, i.e., differing by only 1 or 2 peaks, with *R. argillacea sensu stricto* AU 080618744-01/3 and AU 125955913-01/3 isolates collected from distinct sputum samples from patient P24. (C) Distinct profiles differing by at least 3 peaks with *R. piperina* AU 110334223-01 and AU 080120123-01 isolates recovered from two distinct patients, P19 and P20.

was identified from two siblings followed up in Paris-Necker hospital (P4 and P5), and all of the respective isolates (3 and 6 isolates corresponding to 3 and 4 sputum samples) belonged to the same genotype.

Twenty-seven isolates from 5 CF patients and 4 isolates from patient P27 with CGD were identified as *R. argillacea sensu stricto*. One CF patient (P25) was sampled only once. In contrast, two sputum samples were available for another CF patient (P23), and rep-PCR analysis of the corresponding isolates revealed the same genotype in the two samples. For patient P26, five isolates from successive sputum samples were analyzed and four of them belonged to the same genotype. Another example of genotype variation over time is provided by patient P24 (Fig. 3). For this patient, 18 isolates corresponding to 10 sputum samples were analyzed. Distinct genotypes were identified from the first culture-positive samples, but only the last one (genotype 4) was really developing in the respiratory tract, causing a chronic colonization of the airways attested by the regular detection of this genotype during a 9-month period. Another genotype then was detected (genotype 1) which progressively became dominant (10 out of the 18 isolates), leading finally to the eradication of genotype 4. One genotype (genotype 3) was shared by two out of the five CF patients who were followed up in geographically distant hospitals. Genotype 7, which was identified in the three samples from patient P27 with CGD, was not detected from CF patients.

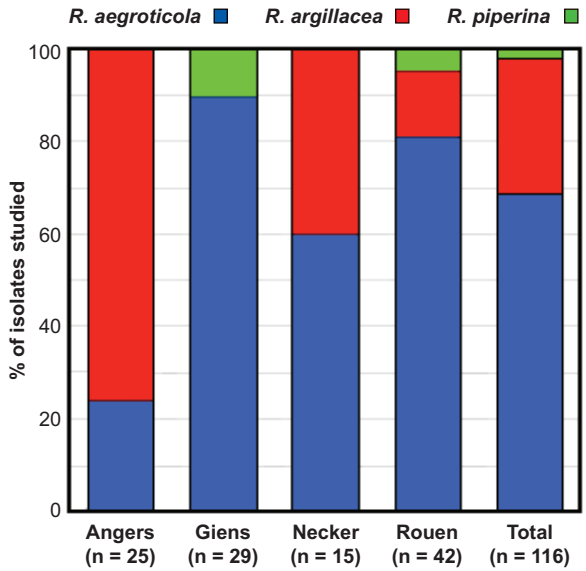

FIG 2 Species distribution within the *R. argillacea* species complex according to the geographic origin of studied isolates. Two isolates were available for one of the patients followed up in Angers Hospital, and their analysis revealed distinct species.

Rasamsonia piperina was identified from three patients (Table 1). A total of five isolates were typed by rep-PCR, and they all belonged to different genotypes. A unique isolate was available for two of the patients, while three isolates from successive culture-positive samples were analyzed for the third patient (P20). rep-PCR analysis of these successive isolates revealed three different genotypes, suggesting a repeated but transient carriage of always distinct genotypes unable to establish within the respiratory tract.

Genotypes restricted to a single patient were found in 7/19, 5/6, and 3/3 patients colonized by *R. aegroticola*, *R. argillacea sensu stricto*, and *R. piperina*, respectively. Two of the species identified in this study, i.e., *R. aegroticola* and *R. argillacea*, were found to be capable of chronically colonizing the lower airways of CF patients, with the same genotype being detected for some patients over a 5- to 11-year period. Six of the genotypes identified for *R. aegroticola* were shared by distinct patients (particularly genotypes 15 and 16, which were detected from 6 and 5 patients, respectively), while all but one *R. argillacea* genotype were patient specific.

In addition, seven genotypes were detected from different locations, for example, the same genotype (genotype 19) was identified from patients followed up in Giens and Rouen hospitals and also in the United Kingdom and Greece.

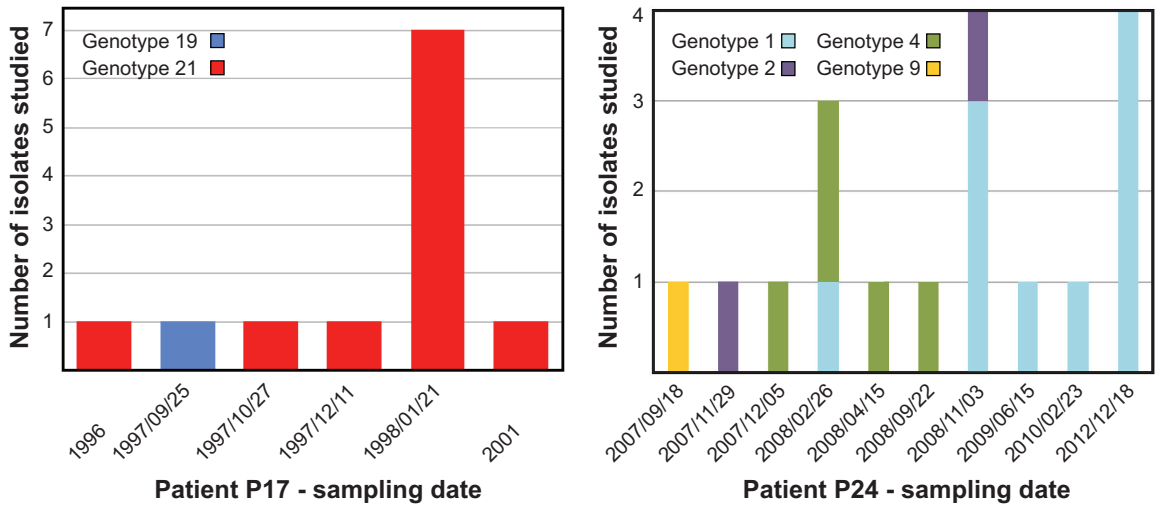

FIG 3 Colonization pattern of two sequentially sampled CF patients showing a largely dominant genotype associated with one genotype found only once (patient P17) or two distinct genotypes successively causing a chronic colonization (patient P24). Genotypes are represented by distinct colors.

DISCUSSION

Since its first description in humans as *Penicillium emersonii* (2), then as *Geosmithia argillacea* (12, 13), and finally as *R. argillacea* (1), the number of case reports involving the *R. argillacea* species complex is dramatically increasing in patients with CF or CGD. Accordingly, 24 out of the 27 patients included in the present study were diagnosed since 2005 and 15 of them since 2010. Nevertheless, nothing is known about the epidemiology of the colonization of the airways by these fungi. In addition, precise species identification within this complex remains difficult: these fungi are not included in the databases used for matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (24); therefore, precise identification of these species still requires the sequencing of discriminant regions in the fungal genome. In the present study, a large set of clinical *R. argillacea* (*sensu lato*) isolates was investigated using the semiautomated DiversiLab system based on the analysis of repetitive DNA sequences. rep-PCR has been previously used successfully for species and/or strain differentiation in several fungal groups, including *Candida* and *Aspergillus* species, as well as dermatophytes and some dimorphic fungi (25, 26, 27, 28, 29). Recently, Matray et al. (23) showed that this method also allows species identification within the *Scedosporium apiospermum* species complex, another group of sibling species encountered in patients with CF, as well as in CGD patients. rep-PCR analysis of our set of isolates revealed a clustering consistent with data from ITS or beta-tubulin sequencing, thereby demonstrating that this method is also suitable for species identification within the *R. argillacea* complex.

As previously reported, only three species of the complex were identified from respiratory secretions of CF patients, i.e., *R. aegroticola*, *R. argillacea*, and *R. piperina*, but not *R. eburnea*. As previously reported (8), very few samples revealed *R. piperina*. However, in contrast to data from Houbraken et al. (8), *R. aegroticola* was largely more common than *R. argillacea sensu stricto* in the present group of CF patients. Isolates from one CGD patient were also analyzed, showing an infection caused by *R. argillacea sensu stricto*. Strikingly, in all cases of *Rasamsonia* infections previously reported in CGD patients since 2000 (9, 10, 11), all species of the *R. argillacea* complex were found except for *R. eburnea*, which is known today only from the environment. All of these species are very close genetically, and therefore it is likely that they share several similar features in their physiology and biology. Since the sequence of the whole genome of two *Rasamsonia* species (*R. emersonii* and *R. byssochlamydoides*) is now available, studies consisting of sequencing the genome of several strains of each species of this complex and of a comparative bioinformatic analysis of the obtained data should be conducted to search for genes common to the three pathogenic species but absent from *R. eburnea* and therefore potentially involved in pathogenicity. A similar approach is currently being used for species of the *S. apiospermum* complex, since all but one species of this complex, *S. dehoogii*, have been described in the CF context (22), although *S. dehoogii* was shown to be one of the most abundant species in the environment (30, 31).

Present data provide the first description of an association of different *Rasamsonia* species in the respiratory tract of a CF patient and of sequential variations of *Rasamsonia* genotypes in the CF lung. Some genotypes of *R. argillacea* and *R. aegroticola* were found persisting for months or years in sequentially sampled CF patients, suggesting that these species chronically colonize the lower airways of CF patients. The capacity of *R. piperina* to chronically colonize the airways was not demonstrated here, but there were only a limited number of CF patients positive for this fungus and very few isolates available for each positive patient. In addition, the influence of prior antifungal therapies and/or of bacterial or other fungal pathogens previously colonizing the airways or causing a respiratory infection was not documented in the present study.

Pathogenicity of the *R. argillacea* complex in CGD patients was unambiguously established, since most patients suffered from pneumonia and/or invasive infection of adjacent tissues (9, 10, 11). In contrast, it seems more limited in CF, with a unique case of respiratory infections caused by this species complex having been reported for this genetically inherited disease (14). Nevertheless, the present identification of some genotypes chronically colonizing the lower airways of patients with CF demonstrates the persis-

tence of these usually saprophytic fungi in the respiratory tract and their multiplication, suggesting their contribution to the inflammatory reaction which progressively leads to clinical and functional deterioration, as demonstrated for long-term colonization of the airways by *Aspergillus fumigatus* or *Candida albicans* (32, 33, 34).

Among the 116 isolates studied here, 29 genotypes were identified. Interestingly, while most genotypes were patient specific, some genotypes were shared by different patients. Six out of the 15 *R. aegroticola* genotypes were shared by epidemiologically unrelated patients living in different geographical areas. Three genotypes were detected from 5 or 6 patients, suggesting a higher capability to colonize the lower airways of the patients. Alternatively, one may also consider a wider distribution or a higher frequency of these genotypes in the environment. Nevertheless, although a few strains have been described from environmental sources, little information is available regarding the ecology of these fungi. Inhalation of some airborne spores has been suggested, but the sources of contamination of the patients remain to be defined.

In addition, two siblings followed up in the Paris-Necker hospital were found to be colonized by the same genotype, suggesting patient-to-patient transmission or contamination through a common source from their environment. Patient-to-patient transmission between siblings with CF or acquisition of the same strain from an identical environmental source within the family situation has been demonstrated for many bacterial pathogens, including methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans* (35, 36, 37, 38). Likewise, concordance in the genotypes colonizing the respiratory tract of siblings suffering from CF has also been reported for some fungal pathogens, like *Candida* species (39) and *Pneumocystis jirovecii* (40). Investigations should be conducted in order to clarify the ecology of these fungi and to identify potential niches in the indoor environment of the patients.

In conclusion, rep-PCR was revealed to be a useful tool for both species identification and strain delineation within the *Rasamsonia* argillacea complex. However, the number of genotypes detected using the present technique may be underestimated, and other typing systems, like multilocus sequence typing or microsatellite marker analysis, should be developed for *Rasamsonia* species in order to confirm our results. Nevertheless, the present study provides the first description of the molecular epidemiology of airway colonization by species of the *R. argillacea* complex in CF patients and demonstrates sequential genotype variations in long-term colonized patients.

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